An outbreak of multidrug-resistant *Acinetobacter baumannii* associated with bronchofiberscopy in an intensive care unit in Beijing, China

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Running Head: Infection related to bronchofiberscopy

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Abstract

Background: As a widely used procedure for diagnosis of various pulmonary diseases in intensive care units, bronchofiberscopy been reported to be associated with nosocomial infections. Between August and December 2009, an outbreak caused by multidrug-resistant Acinetobacter baumannii (MDR-Ab) was observed in an ICU of a tertiary care hospital in Beijing, China. This study is aimed to describe the course and control of this outbreak and investigate the related risk factors.

Methods: This case-control study was performed, including case identification, medical record review, environmental cultures and genotyping with repetitive extragenic palindromic PCR (REP-PCR).

Results: Twelve patients infected or colonized with MDR-Ab were included. Seven who met the case definition were compared with 19 controls. Six of the seven cases (83%) were treated with bronchofiberscopy versus four of the 19 controls (21%) (Odds ratio, 22.5; 95% confidence interval, 2.07-244.8; P=0.005). Sixteen (72.7%) of 22 MDR-Ab isolated from seven cases and 22 (84.6%) of 26 MDR-Ab isolated from bronchofiberscopy and healthcare-associated environment were clustered significantly into a major clone when analyzed by REP-PCR.

Conclusions: Bronchofiberscopy was shown to be a significant risk factor for the acquisition of MDR-Ab. Infection control precautions, which include appropriate bronchofiberscope reprocessing and environmental decontamination, should be strengthened.

Keywords: Outbreak; bronchofiberscopy; multidrug-resistant Acinetobacter baumannii
Introduction

Multidrug-resistant *Acinetobacter baumannii* (MDR-Ab) has become one of the most important healthcare-associated pathogens worldwide and causes infections such as hospital-acquired pneumonia, wound infection, meningitis, endocarditis and bloodstream infection (BSI) due to its prolonged environmental survival and extensive resistance to many of the antibiotics, that are commercially available such as cephalosporins, aminoglycosides, quinolones, and carbapenems.\(^1\) Nosocomial MDR-Ab infection most likely occurs in intensive care units (ICUs), although epidemic strains have also been isolated in other hospital departments.\(^2,3\) Outbreaks of MDR-Ab in ICUs have been reported to be associated with various types of devices and medical procedures used in patient management.\(^4,5\) Most relevant reports refer to medical devices and procedures used for respiratory systems, such as mechanical ventilators, laryngoscope blades and tracheostomy.\(^6-8\) In addition, long hospital or ICU stays, exposure to infected or colonized patients in neighboring hospital environment, critical illness and administration of broad-spectrum antimicrobial agents are very important for transmission of MDR-Ab in institutions during outbreaks.\(^9-12\) Outbreaks of blood stream infection caused by MDR-Ab have also been reported and the clinical manifestations of MDR-Ab bloodstream infection may range from transient bacteriemia to septic shock and fulminating disease accompanied with an overall mortality (case-fatality ratio) as high as 46%.\(^13-15\)

Bronchofiberscopy is the visual examination of the tracheobronchial tree through a fiberoptic bronchofiberscope and is currently an indispensable tool used in the ICUs. Several nosocomial infections caused by *Pseudomonas aeruginosa*, *Serratia marcescens* and *Mycobacteria*, etc. had been reported to be associated with bronchofiberscopy and the reprocessing of bronchofiberscope, such as departure from cleaning and disinfection procedures,\(^16\) problems related to bronchoscopy suite,\(^17-19\) and device defects (e.g. a loose
biopsy port cap, damage due to long physical use). 20-22 However, to date, there is no report about the involvement of bronchofiberscopy in outbreaks of MDR-Ab. In September 2009, the Center for Hospital Infection Control & Research, Institute for Disease Control & Prevention of PLA, China received a report from an ICU in a 1200-bed hospital that a cluster of five patients have healthcare-associated bloodstream infection caused by MDR-Ab. The patients had different primary diagnoses and six of seven clustered cases had received bronchofiberscopy. This report is the first one that describes a nosocomial MDR-Ab outbreak related to bronchofiberscopy.
Methods

Ethics Statement

The study was approved by the Institutional Ethic Committees of the Academy of Military Medical Sciences and 309 hospital of the Chinese People’s Liberation Army, Beijing, China. The written consent from all the patients involved in the study was obtained before the study.

Setting

The hospital in which the outbreak occurred was a 1 200-bed tertiary care center in Beijing. The outbreak occurred in an ICU ward for adult patients that was composed of a large open bedroom with 10 beds, a buffer room, treatment room, doctors’ office and a room for equipment. Every bed was equipped with an alcohol-based hand rub. Patients from departments of both surgical and internal medicine were admitted into the ICU. In total, 15 doctors and 31 nurses worked in this ICU and approximately 12 nurses were on duty every day. The workload is very high. There was only one bronchofiberscope in the ICU and the bronchofiberscopy was performed one or two times each day for diverse examination and treatment such as corpus alienum removal, clearance of secretion, tracheal intubations and bronchoalveolar lavage, etc. After each procedure the bronchofiberscope was normally reprocessed by the professional staffs in the center for cleaning and disinfection in the hospital according to Chinese Guidelines for Endoscopy Cleaning & Disinfection. The standard procedure for reprocessing bronchofiberscope includes the following steps: precleaning, cleaning with an enzymatic detergent, rinsing, disinfection (for 20 minutes in a 2% glutaraldehyde solution), final rinsing, drying and storage. However, during the outbreak time the bronchofiberscope was reprocessed directly and manually by the doctor who had used it in the ICU after each bronchofiberscopy when it was emergently and frequently
needed for patients. Neither doctor nor nurse was appointed specifically for reprocessing the bronchofiberscope and no automatic reprocessing machine was used.

**Epidemiological Investigation**

Medical records were reviewed that included paper and electronic charts. Microbiological records were analyzed carefully to screen the cases and define the baseline rate of MDR-Ab before the outbreak. Any patient who had at least one clinical or screening sample that was positive for a MDR-Ab and with the corresponding clinical symptoms (such as pneumonia, bacteriemia and peritonitis) detected at least 48 h after ICU admission was noted. Multidrug resistance was defined as resistance to three or more classes of drugs used for treatment of acinetobacter infections (e.g., quinolones, cephalosporins and carbapenems). Environmental samples were taken from the hands, nasal cavity of the staff in the ICU and multiple surfaces in the environment of the ICU, which included bed sheets, bedrails and bed tables associated with case patients and controls; work clothes, computer keyboards and calculators of healthcare workers; the surfaces of medical equipments such as invigilator, ventilator, hemofiltration machine, bronchofiberoscope, electrocardiograph machine, ultrasonic machine and laryngeal endoscopes. Environmental sampling was done three times on 15th, 21th and 28th October 2009, respectively.

**Case-Control Study**

The case-control study was conducted to investigate the risk factors of this outbreak. A case was defined as any patient who had the outbreak strain of MDR-Ab in a clinical culture that had been taken at least 48 h after ICU admission between August 2009 and January 2010. The clinical cultures included urine, sputum, wound, or blood during the study period. The MDR-Ab infection was defined as the MDR-Ab positive in clinical cultures from sterile
sites, like urine, catheter, or blood; whereas MDR-Ab colonization was referred to the MDR-Ab positive in clinical cultures from non-sterile sites, like sputum. Controls were patients without MDR-Ab infection or colonization who stayed in the same ICU for more than 48 h during the same period. The patients who stayed in the ICU for less than 48 h, patients who were infected with strains other than the outbreak strain as determined by REP-PCR and patients who had been admitted to the ICU already harboring MDR-Ab were all excluded. The ratio of controls to cases was 2.7 to 1. Every control had at least one clinical culture of urine, sputum, wound, or blood during the study period. For the case-control study, the following data were collected: demographic data (including age, sex), hospital days, days in ICU, blood transfusion, mechanical ventilation, bedside diagnostic ultrasound, bedside chest X-ray, bronchofiberscopy, electrocardiogram, venipuncture, gastric lavage, urinary catheterization, hemodialysis, presence of a central line, surgical operation, antibiotic usage, and primary disease including septic shock, multiple organ failure, pulmonary diseases and renal diseases.

Microbiological Methods

The samples were inoculated in the blood agar and the isolates were identified by the automated Microscan-Walkaway Microbiology Identification System (Becton Dickinson, USA). Antimicrobial susceptibility was determined by the disk diffusion method and interpreted according to Clinical Laboratory Standards Institute (CLSI) guidelines on the following antibiotics: cefuroxime, cefotaxime, cefepime, imipenem, amikacin, minocycline, levofloxacin, and colistin. DNA extractions of isolates were typed by REP-PCR using REP1 (5′-IIIICGICGCATCIGGC-3′) and REP2 (5′-ICGTATCIGGCCTAC-3′) primer sequences.24 Characteristic DNA patterns were analyzed using the BioNumerics software (version 3.0, Applied Math, Sint-Martens-Latem, Belgium) to determine the distance
matrices and the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) method to create a dendrogram.

Intervention

Three major interventions were implemented. First, the reprocessing procedure for the bronchofiberscope by doctor in the ICU was stopped and the bronchofiberscope was sent to the center for cleaning and disinfection for professional reprocessing. Another one or two bronchofiberscopes were organized to support under emergency situation in ICU. Second, the surveillance culture for MDR microorganisms from the bronchofiberscope was performed regularly after every reprocessing. Third, environmental surfaces in the ICU were cleaned thoroughly and disinfected with disinfectant containing HCLO (electrolyzed acid water) according to the manufacturer’s instruction. Forth, education and training were enhanced for endoscopy reprocessing and general infection control procedures in this ICU. In addition, to investigate the effect of hand hygiene on this outbreak, hand hygiene compliance of healthcare workers was observed as described previously. Briefly, infection control professionals recorded opportunities for hand hygiene during 1-hour observation periods distributed randomly between 8:00 a.m. and 5:00 p.m. every day during the investigation. Hand hygiene compliance prior to the outbreak was calculated by review of the video document in the ICU.

Statistical Analysis

Statistical analyses were performed using SAS software (SAS Institute Inc., version 8.1). In case-control study, cases and controls were compared by Mann-Whitney \( U \)-test or Student’s \( t \)-test for continuous variables and by Fisher’s exact test for categorical variables. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated for binomial variables. A
two-sided $P$-value of less than 0.05 was considered statistically significant. Multivariate logistic regression analysis was not applied because of small sample size.
Results

Epidemiologic Investigation

The average number of healthcare-associated MDR-Ab for the two years prior to the outbreak was two cases per half a year. There were only three healthcare-associated cases of MDR-Ab between January 2009 and the beginning of the outbreak in September 2009. A total of 12 patients (7 male and 5 female, aged from 39 to 97) were found to be infected (8 patients) or colonized (4 patients) with MDR-Ab at least 48 h after ICU admission from 5th August 2009 to 30th December 2009 and the number of cases of MDR-Ab was higher than the usual incidence (Figure 1, Figure 2 and Table I). Two culture-positive patients who were admitted before 20th September 2009 into the ICU were found by review of the medical records. Five cases were identified in 18 days between 21th September and 8th October 2009, and three other cases were found in four days between 19th October and 22th October 2009. Thereafter, only two cases were identified on 2nd November and 30th November and no other case was detected up to the end of June 2010.

The patients had a variety of underlying conditions that included septic shock, organ transplantation, malignant tumor, respiratory failure, abdominal infection, acute pancreatitis, chronic obstructive pulmonary disease, multiple organ dysfunction syndrome, coronary heart disease, etc. Seven of the 12 patients were identified when they were staying in beds nos. 1, 2, 3, 6, 7 and 8, as shown in Figure 3. The average interval between ICU admission and the date of MDR-Ab identification was 6.3±3.8 days (data not shown). Eight of the 12 patients had received bronchofiberscopy and five had bloodstream infection. Six patients (50%) died in the ICU and three patients’ deaths (B, D, E) were possibly related to MDR-Ab infections (Table I).

In total, 22 MDR-Ab isolates were available from seven patients who had undergone bronchofiberscopy and from four patients who did not have bronchofiberscopy. All the
MDR-Ab isolates from patients were obtained from sputum, blood, catheter, ascites, pleural fluid, bile and wounds and the isolates were completely resistant to cefuroxime, cefotaxime, imipenem and levofloxacin but susceptible to colistin. By REP-PCR analysis it was shown that 16 MDR-Ab isolates from six patients who received bronchofiberscopy showed one identical strain of MDR-Ab (genotype A) that was different from isolates (genotype C, D, G) obtained from three patients who did not have bronchofiberscopy (Table I). In the present study this genotype A MDR-Ab was defined as outbreak strain. Patient F did not receive bronchofiberscopy, but had the outbreak strain of MDR-Ab in her bile and catheter. Patient K received bronchofiberscopy after intervention and an unrelated MDR-Ab (genotype B) was detected in the sputum. In addition, these 16 outbreak MDR-Ab strains from the six patients who had undergone bronchofiberscopy were associated with nos.1-3 and nos. 6-8 beds (Figure 3).

Meanwhile, 33.3% (26/78) of environmental samples were MDR-Ab-positive and 22 of these 26 MDR-Ab-positive strains (84.6%) were identical to the outbreak MDR-Ab strain from the patients. In these 22 strains, four were recovered from the biopsy forceps and the tip of the bronchofiberscope that was used to treat one case patient, and from the surface of bronchofiberscope after reprocessing in the ICU. Thirteen strains were recovered from the bedside tables, bed sheets, and bedrails of nos. 1-3 and nos. 6-8 beds, which was in line with the distribution of outbreak strains from patients. Five strains were detected in the nursing station, the nurse work clothes, the scrub sink and the medical treatment room (Figure 3). However, no culture of samples from the hands and nasal cavity of healthcare workers was positive for MDR-Ab (data not shown).

Further investigation disclosed several potential administrative and technical problems in bronchofiberscope reprocessing. First, from the end of July of 2009 the bronchofiberscope was frequently reprocessed in the ICU by doctors under emergency for patient’s examination
and treatment. Second, the reprocessing procedure of the bronchofiberscope was not strictly in accordance with the Chinese Guidelines for Endoscopy Cleaning & Disinfection. For instance, the pre-cleaning time was not adequate and the specific detergent containing enzyme was seldom used. In addition, the patients who receive bronchofiberscopy was seldom covered during the emergent treatment and after bronchofiberscopy the possible contaminated environmental surface was not disinfected immediately and thoroughly.

Case-Control Study

Seven cases were identified according to the definition given. There were five other patients who did not meet the definition; out of these five, one did not have a strain available for genotyping and four had MDR-Ab strains that differed from the outbreak strain. The cases and controls were similar with respect to age and sex (Table II). The mean number of hospital days was greater for the cases than for the controls (42 days versus 31 days), but the difference was not statistically significant. Univariate analysis confirmed that bronchofiberscopy was a significant risk factor for acquisition of MDR-Ab. Six (85.7%) of seven cases versus four (21.1%) of 19 controls were treated with bronchofiberscopy. Case-control analysis indicates an odds ratio of 22.5 (95% CI, 2.07-244.84; P =0.005). The case patients had more septic shock than the controls (4 of 7 versus 1 of 19, P = 0.01), indicating more serious underlying disease in the cases than in the controls. Five of seven case patients versus one of controls were associated with administration of carbapenems (OR, 45; 95% CI, 3.35-603.99; P =0.002). Another significant factor was number of days spent in the ICU (median, cases versus controls, 26 versus 3; P <0.001). All other variables tested, which included mechanical ventilation, presence of central line, pulmonary diseases, blood transfusion and fluoroquinolones administration, were not different significantly between case patients and controls (Table II).
Intervention

Intervention was implemented on 21\textsuperscript{th} October 2009. Follow-up of environmental cultures of surfaces in the ICU has been performed monthly for 6 months and none had grown MDR-Ab. Hand hygiene compliance of healthcare workers rose from 30\% to 90\%, especially hand hygiene before patient contact which was raised from 27.5\% to 86.9\%. MDR-Ab cases decreased gradually and no further cases of outbreak strain were detected in December.
Discussion

The present study describes the first recognized nosocomial outbreak of *A. baumannii* associated with the bronchofiberscope. Seven of 12 patients during the outbreak period were infected or colonized with a single strain of MDR-Ab that was also cultured from multiple environmental surfaces in the ICU. Only one patient acquired the outbreak strain of MDR-Ab without known exposure to bronchofiberscopy treatment. Although the emergence of MDR-Ab is becoming more frequent in Chinese hospitals, a localized nosocomial outbreak is reported rarely in China.

The association of bronchofiberscopy with this outbreak highlights the importance of appropriate infection control measures when this invasive medical procedure is performed. In this study we have provided further evidence that MDR-Ab was disseminated during the bronchofiberscopy examination thoroughly in the ICU during the outbreak periods and was associated significantly with case patients. Eighty-five percent of the MDR-Ab isolates from the environmental samples were identical to the outbreak strain, indicating the serious contamination of the surrounding environmental surfaces. Importantly, four outbreak strains were isolated directly from the non-disinfected and disinfected bronchofiberscope, which suggested that the serious failure existed in reprocessing the used bronchofiberscope and the outbreak strain of MDR-Ab might have been transmitted directly through bronchofiberscopy treatment. Alternatively, they could have been introduced into the environment by one of the case patients, or possibly, by an unidentified patient, then transmitted through the hands of healthcare workers or other medical procedures. In the investigation, most of the environmental MDR-Abs were isolated from the healthcare workers-related environmental surfaces, such as bed sheets, bedrails, nurse desks, computer keyboards, and outer surface of the invigilator. However, there was no positive culture of samples from the hands and nasal cavity of healthcare workers (data not shown), which might be associated with high hand
hygiene compliance during the investigation because all the healthcare workers were concerned of the probable personnel correlation with this outbreak of MDR-Ab.

In addition to bronchofibroscopy treatment, the case-control study by univariate analysis showed that septic shock was more common in the case patients than in the controls. Similar results were also found that the underlying severity of illness of the patients was a significant factor contributing to the acquisition of carbapenem-resistant A. baumannii in the ICU.\textsuperscript{28} Moreover, days in the ICU and carbapenem administration were also identified as risk factors.\textsuperscript{29,30} Attempts were made to identify independent risk factors by multivariate logistic regression, however, the sample sizes were too small to allow reliable conclusions.\textsuperscript{5}

The bronchofiberscopy is used increasingly for diverse examinations and treatments in ICUs. Our findings emphasize that bronchofiberscopy must be performed under controlled conditions with appropriate infection control measures in place. The present outbreak is not associated with defects and damage of the bronchofiberscope,\textsuperscript{20-22} but apparently associated with the cleaning and disinfection procedures. It was not occasional that the bronchofiberscope was reprocessed in ICU when it was urgently needed for clinical examination for patients; however, strict reprocessing of bronchofiberscopes should be performed after each procedure and at the end of the day following the guidelines. It might be a good solution to increase the number of bronchofiberscope in ICU to guarantee the professional reprocessing of the bronchofiberscope in the Center for Cleaning and Disinfection of the hospital; however, it is usually limited by economic reason, especially in the less-developed districts or countries. Therefore, assigning and training certain personnel for strict reprocessing of bronchofiberscope in the ICU might be also plausible solution. On the other hand, at least standard precautions have to be implemented during bronchofiberscopy, such as use of personal protective equipment, which includes fluid-resistant gowns, gloves, surgical masks, eye protection, and shoe and hair covers.\textsuperscript{30} In
addition, the patients who receive bronchofiberscopy should be covered during the treatment and the possible contaminated environmental surface should be cleaned thoroughly and disinfected.

This outbreak was clinically significant due to the extensive antibiotic resistance of A. baumannii and the severity of the patient outcomes. Five of six cases who received bronchofiberscopy treatment developed MDR-Ab BSI with severe clinical manifestations. Half of the number of patients died in the ICU during the outbreak periods and MDR-Ab infection possibly contributed to three deaths. However, the significance of this case-control study was limited by the small sample size and wide 95% CIs. The results of this case-control study demonstrate an association between factors but cannot lead to conclusions regarding to causality. Further studies of outbreaks similar to this one are needed to confirm the results. However, a strong association between bronchofiberscopy and MDR-Ab acquisition was fortified by the epidemiologic and microbiologic studies conducted during the outbreak.
Author’s Contributions

LH conceived of the study, revised and approved the manuscript. CL participated in its design and coordination and helped to draft the manuscript. YX carried out the microbiological study and draft the manuscript. YC contributed to data collection and analysis and interpretation. FW participated in the environmental sampling. BY participated into the study design. JZ contributed to the clinical document collection and data analysis. GJ carried out the molecular typing. XH participated in the sampling for endoscopy. XD revised the manuscript for important intellectual content. ZW joined the design of the study and performed the statistical analysis. All authors read and approved the final manuscript.

Acknowledgments

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Conflict of interest statement: None declared.
Reference List


Table I. Clinical characteristics of multidrug-resistant *Acinetobacter baumannii* (MDR-Ab) infected/colonized patients from 5\(^{th}\) August 2009 to 31\(^{th}\) January 2010 in ICU

<table>
<thead>
<tr>
<th>Patients</th>
<th>MDR-Ab culture sites</th>
<th>Bronchofiberscopy</th>
<th>Patient outcome</th>
<th>MDR-Ab strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Sputum</td>
<td>Yes</td>
<td>Survival</td>
<td>NA</td>
</tr>
<tr>
<td>B</td>
<td>Ascites, sputum</td>
<td>No</td>
<td>Died</td>
<td>G</td>
</tr>
<tr>
<td>C</td>
<td>Sputum, blood</td>
<td>No</td>
<td>Survival</td>
<td>C</td>
</tr>
<tr>
<td>D</td>
<td>Blood, sputum, catheter</td>
<td>Yes</td>
<td>Died</td>
<td>A</td>
</tr>
<tr>
<td>E</td>
<td>Blood, sputum, pleural fluid</td>
<td>Yes</td>
<td>Died</td>
<td>A</td>
</tr>
<tr>
<td>F</td>
<td>Bile, catheter, sputum,</td>
<td>No</td>
<td>Survival</td>
<td>A</td>
</tr>
<tr>
<td>G</td>
<td>Blood, sputum, catheter</td>
<td>Yes</td>
<td>Died</td>
<td>A</td>
</tr>
<tr>
<td>H</td>
<td>Blood, sputum, catheter</td>
<td>Yes</td>
<td>Died</td>
<td>A</td>
</tr>
<tr>
<td>I</td>
<td>Sputum</td>
<td>Yes</td>
<td>Survival</td>
<td>A</td>
</tr>
<tr>
<td>J</td>
<td>Blood, sputum, wound</td>
<td>Yes</td>
<td>Survival</td>
<td>A</td>
</tr>
<tr>
<td>K</td>
<td>Sputum</td>
<td>Yes</td>
<td>Died</td>
<td>B</td>
</tr>
<tr>
<td>L</td>
<td>Sputum</td>
<td>No</td>
<td>Survival</td>
<td>D</td>
</tr>
</tbody>
</table>

MDR-Ab, multidrug-resistant *Acinetobacter baumannii*; NA, isolate not available for analysis.
Table II  Comparison of selected risk factors for healthcare-associated infection or colonization with multidrug-resistant *Acinetobacter baumannii* during the outbreak period in the ICU, from 5\(^{th}\) August 2009 to 31\(^{th}\) January 2010.

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>No. (%)</th>
<th>95% CI</th>
<th>OR (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y (mean±SD)</td>
<td>67.1±22.9</td>
<td>67.2±16.9</td>
<td>.99</td>
<td>-</td>
</tr>
<tr>
<td>Male</td>
<td>5(71.4)</td>
<td>12(63.2)</td>
<td>1.00</td>
<td>1.46(0.22-9.62)</td>
</tr>
<tr>
<td>Hospital stay, days [median(IQR)]</td>
<td>42(11-82)</td>
<td>31(8-71)</td>
<td>.24</td>
<td>-</td>
</tr>
<tr>
<td>ICU stay, days [median(IQR)]</td>
<td>26(10-52)</td>
<td>3(2-6)</td>
<td>&lt;.001</td>
<td>-</td>
</tr>
<tr>
<td>Bronchofibroscopy</td>
<td>6(85.7)</td>
<td>4(21.1)</td>
<td>.005</td>
<td>22.50(2.07-244.84)</td>
</tr>
<tr>
<td>Mechanical ventilation</td>
<td>5(71.4)</td>
<td>15(79.0)</td>
<td>1.00</td>
<td>0.67(0.09-4.81)</td>
</tr>
<tr>
<td>Presence of central line</td>
<td>3(42.9)</td>
<td>5(26.3)</td>
<td>.64</td>
<td>2.10(0.34-12.86)</td>
</tr>
<tr>
<td>Septic shock</td>
<td>4(57.1)</td>
<td>1(5.3)</td>
<td>.01</td>
<td>24.00(1.95-295.06)</td>
</tr>
<tr>
<td>Pulmonary diseases</td>
<td>6(85.7)</td>
<td>8(42.1)</td>
<td>.08</td>
<td>8.25(0.82-82.67)</td>
</tr>
<tr>
<td>Blood transfusion</td>
<td>6(85.7)</td>
<td>13(68.4)</td>
<td>.63</td>
<td>2.77(0.27-28.39)</td>
</tr>
<tr>
<td>Fluoroquinolones administration</td>
<td>3(42.9)</td>
<td>3(15.8)</td>
<td>.29</td>
<td>4.00(0.58-27.82)</td>
</tr>
<tr>
<td>Carbapenems administration</td>
<td>5(71.4)</td>
<td>1(5.3)</td>
<td>.002</td>
<td>45.00(3.35-603.99)</td>
</tr>
</tbody>
</table>

CI, confidence interval; ICU, intensive care unit; IQR, interquartile range; OR, odds ratio; SD, standard deviation; -, not measured.
Figure 1. Epidemical curve that shows the rate of healthcare-associated multidrug-resistant *Acinetobacter baumannii* (MDR-Ab) in the ICU in 2009. The outbreak period was from September to October 2009, when a marked increase in the number of cases was noted. The pattern indicates various strains of MDR-Ab as defined by REP-PCR. After the outbreak was halted in late October, the number of cases reduced. However, there were still two patients who acquired MDR-Ab in November. The strains from these two patients were unrelated to the outbreak strain. In December and the next January no healthcare-associated MDR-Ab was detected.

Figure 2. Timeline of 12 patients with healthcare-associated multidrug-resistant *Acinetobacter baumannii* (MDR-Ab) infection during the outbreak. This timeline depicts the 12 patients who were identified as infected or colonized with MDR-Ab from August to December 2009. Patient’s duration in the intensive care unit (ICU), use of bronchofiberscopy, the positive culture of unrelated strains or outbreak strains is indicated in the figure. Asterisks indicate case patients.

Figure 3. Schematic map of ICU and the distribution of outbreak multidrug-resistant *Acinetobacter baumannii* (MDR-Ab) strain and other MDR-Ab strains identified in the outbreak. Numbers 1-10 indicate the bed number (rectangles) in the ICU. Black dots represent the outbreak strain and white letters inside are the patient numbers. Gray dots represent the outbreak strains isolated from the environment. The black square represents unrelated MDR-Ab strains from patients. The ellipse indicates the sink for hand washing. Dashed lines indicate physical barriers by drapes. There was two meters distance between the adjacent beds.
Figure 4. Cluster analysis of multidrug-resistant *Acinetobacter baumannii* (MDR-Ab) isolates by REP-PCR. Forty-eight isolates of MDR-Ab were classified into eight genotypes according to 90% similarity by REP-PCR. Among these, 38 isolates inside the long panel demonstrate type A, which was found to be the major type. The other isolates were not identical and corresponded to other types. A percent genetic similarity scale is shown above the dendrogram. Both band position tolerance and optimization were set at 2.0%.
Figure 2